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(57) Abstract

A method for the decrease of fat absorption in any animal, wherein the animal is fed an antibody produced against lipase, an enzyme which is required for fat absorption.

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DECREASED FAT ABSORPTION WITH AN ANTI-LIPASE ANTIBODY

A food additive that decrease fat absorption in mammals

Our sedentary life including the decreased physical activity and increased food intake have made us prone to be overweight. The above has brought as consequence that almost 40-50% of the USA population is 20% above their desirable weight. The advance in the science of food and nutrition not only has made us wiser about the functions of all nutrients but also by applying that knowledge we have concentrated food in smaller portions by which the satisfaction of filling is decreased. Even if the amount of food intake remains the same, we will have an excess caloric intake due to the high energy concentration of such type of food (Bell, et al 1997). Currently, the weight loss related market is full of diet pills that reduce appetite by suppressing brain hormones, drugs that suppress the absorption of nutrients, pills that supposedly have ergogenics effect, pills that increase food passage rate and other fad diets. Mostly all of these drugs have secondary effects like depression, anxiety, addiction and others.

A new approach for the reduction of calories in food is by the use of fat substitutes (Gershoff, et al 1995). Each gram of fat provides 9 calories as compared to 4 calories per gram of carbohydrate and protein. Fat substitutes mainly those made of long

carbohydrate chains are use for the claboration of prepared food with the purpose of maintaining fat properties in the prepared food but decreasing calories. A new fat substitute, Olestra, which is made of long chain fatty acids that are too big for digestive enzymes (lipase) to breakdown, therefore that type of fat is not absorbed. It has been observed that the consumption of Olestra has resulted in decreased absorption of fat soluble and the presence of fat in the feces. A long term study (12 weeks) where 1/3 of the dietary fat was replaced with olestra, female subjects lost weight and did not compensate for the reduced calories and fat intake (Roy, et al, 1997).

In the animal industry, researches have been working on the reduction of fat accumulation in animals since this characteristic first, has a negative effect on profits and second, consumers want less visible fat in order to decrease the health risk.

Fat accumulation in animals has been reduced by passively administered antibodies against adipocyte plasma membrane in rats, pigs, rabbits and lambs. Immunity against growth hormone has also decreased abdominal fat in chickens (Brodic and Hu, 1996; Moloney, 1995; Flint, 1992).

Lipase, an enzyme produced by the pancreas, hydrolyzes triacylglicerides into free fatty acids and glycerol. This is a crucial step in breaking down ingested fat in the gastro-intestinal tract. Lipase is more active in the duodenum (small intestine) where broken down fat with the aid of bile salts form micelles and then are absorbed by the intestinal mucosa.

Therefore, by inhibiting lipase the ingested fat will not be absorbed and the energy supplied by fat and the fat itself will be excreted.

A method for the inhibition of fat absorption in mammals.

The present invention relates to a method for decreasing fat absorption by orally feeding chicken antibodies against lipase. The preferred antigen for obtaining the antibodies is a swine pancreatic extract that contains lipase. This antigen is commercially produced by Sigma Chemical Co. lipase is a conserve molecule with similar structure between animal and plant species, therefore an antibody against swine lipase will cross-react with other species' lipases. We have found that by feeding anti-lipase antibodies to mice and rats will result in either decreased body weight or reduced feed efficiency. The antibody extract can either be fed in water suspension, included in feed as dry powder and/or encapsulated in liposomes.

Previous research on the effectiveness of chicken antibodies has been reported; i.e. the prevention of bacterial infection in swine, calf and dairy cows (Yokoyama et al, 1993; Erhard et al 1993; Coleman, 1995). These researches have also demonstrated the presence of intact avian antibodies in the gastro-intestinal tract of the animals.

Although chickens antibodies are known to protect against bacterial infections, no antibody has been reported to decreased fat absorption.

It will be apparent for those skilled in the art that the aforementioned objects and other advantages may be further achieved by the practice of the present invention.

Example 1

This example illustrate the preparation of the specific antibody against lipase. 17-week old hens were injected with 2.5 mg of lipase (Sigma Chemical Co.). The inoculum was prepared by dissolving the enzyme in 0.2 ml phosphate buffered saline (PBS, pH 7.3) and 0.2 ml complete Freund's adjuvant. The antigen preparation was injected into two sites 0.2 ml in each (right and left) pectoralis muscle. A total of 0.4 ml of antigen preparation per hen was administered. A second injection was administered 5-6 weeks following the initial injection (at about 50% egg hen production). In the second antigen preparation, incomplete Freund's adjuvant was used instead of complete Freund's adjuvant. Hens were re-injected with the antigen preparation every two months or when the antibody titer was determined to be low. Antibody titer was determined by ELISA. Hens had free access to fed and water and they were maintained in an isolated room in order to minimized outside contamination.

Example 2

Antibody was purified as follows: One volume of egg yolk of example 1 was mixed with 9 volumes of distilled water and left to sit overnight at 4 C°. Then the aqueous portion was contrifuged at 4000 rpm for 10 minutes and filtered through a cheesecloth in order to remove any excess fat. The aqueous portion contains all the protein present in the egg yolk which includes all the antibodies (IgY). The liquid was frozen and then was freeze dried. The antibody activity was determine by ELISA.

Example 3

Antibody against lipase was determined as follows:

1.- ELISA plates were coated with 100 ul lipase preparation (50 ug/ml) in carbonate buffer. The plates were incubated at 4° C overnight prior to blocking with 1.5% bovine serum albumin for 4 hours at room temperature.

2.- 100 ul of a 0.5 mg protein/ml antibody extract was added to each well and the plates incubated at room temperature for 1 hour.

3.- Plates were washed with PBS-tween solution. 100 ul of rabbit anti-chicken IgG conjugated to horseradish peroxidase was added to each well. The plates were incubated at room temperature for 1 hour.

4.- Plates were washed with PBS-tween and 100 ul of TMB substrate was added to each well and incubated for 15 minutes.

5.- The reaction was stopped with 100 ul of 2 M sulfuric acid.

6.- Plates were read at 455 nm in an ELISA plate reader.

7.- Titer was determine as the inverse of the dilution at which O.D. of the immunized egg was similar to the unimmunized control (O.D. < 0.100).

Example 4

This study illustrates the in vitro inhibition of lipase by the chicken anti-lipase antibody. The effectiveness of the antibody was verified by using a test specific for the determination of lipase in serum (Sigma Chemical Co.). We modified this test by adding a known amount of enzyme (lipase) and antibody to a certain volume of phosphate buffered saline. The resulting activity was expressed as Sigma-Tietz units/ml, which is equal to the ml of 0.05 N NaOH required to neutralize the fatty acid formed in the reaction. In a preliminary study we found the following:

Lipase (mg)	anti-lipase (protein extract) (mg)	Lipase Activity (U)	% decreased activity
2.0	0	17.3	
2.0	9.0	18.8	0
1.0	0	14.0	
1.0	9.0	12.5	11
0.5	0	10.4	
0.5	9.0	9.8	6
0.25	0	8.1	
0.25	9.0	6.7	17

In a second test; high amount of antibody extract was used. The results are as follows:

Lipase (mg)	Anti-lipase Protein Extract (mg)	Lipase Activity (U)	% decreased activity
2.0	0	18.7	
2.0	37	14.0	25
1.0	0	13.5	
1.0	37	6.9	49

Example 5

This study illustrates the effect of anti-lipase antibody in mice. Two groups of 5 2-month old mice (25-34 gr each) were given 5 mg of antibody (protein extract) per ml of water. The antibody was mixed with water on a daily basis. Mice were fed the same amount of feed in both groups (approx. 5 gr/mice/day). The length of the experiment was 58 days. The results are as follows

	total* initial body weight (gr)	total final body weight (gr)	difference in body weight (gr)	total feed intake (gr)	gr of feed needed to gain 1 gr of body weight
control	157	199	42	1039	24.74
anti-lipase	156	187	31	1039	33.52

*Sum of 5 mice/trt

Example 6

This study illustrate the effect of anti-lipase when fed at lower levels than in example 5. Two groups of 5 5-month old mice (32-35 gr each) were given 1 mg of antibody (protein extract) per ml of water for the first 7 days then it was increased to 2 mg/ml of water. Mice in both groups were fed the same amount of feed for 35 days. The results were as follows.

	Total initial body weight (gr)	Total final body weight (gr)	difference in body weight	total feed intake (gr)	gr of feed needed to gain 1 gr of body weight
control	182	204	22	787.4	35.79
anti-lipase	181	185	4	788.5	197.13

Example 7

This study demonstrate the encapsulation of anti-lipase antibodies by liposomes. The liposome preparation was based on the procedure by Shimizu et al (1993). Final liposome suspension was frozen and later freeze dried. A known amount of freeze dried liposome was mixed with rat diet, and fed daily for the length of the study.

Example 8

This study illustrate the effect of anti-lipase antibodies in rats. Twelve retired breeder Sprague Dawley rats (Harlan, Wisconsin) were individually caged and supplied with free access to water. They were fed a rabbit chow which was supplemented with corn oil in order increase fat content to 30%. Feed intake was monitored for 1 week in order to determine the amount of feed needed to maintain their initial body weight. Rats were divided into two groups one fed the high fat diet and the other group was fed the same diet with freeze dried liposome containing anti-lipase antibody extract. The treated diet contained 750 mg antibody/kg of diet. The results after 1 week of treatment are as follows:

	Initial body weight (gr)*	One week feed intake (gr)	Final body weight (gr)	grams of feed needed to gain 1 gr of body weight
control	316	132.3	327	12.0
antibody	319	129.4	326	18.5

* average of 6 rats.

Example 9

Since it was observed that rats gained weight in example 8, the same rats were used but this time feed was restricted. The results are as follows:

	initial body weight (gr)	Final body weight (gr)	difference in body weight	feed intake (gr)
control	327	319	-8	102
antibody	326	317	-9	101

Example 10

This study demonstrate the effect of the anti-lipase when fed to rats at maintenance feed intake. The results are as follows:

	initial body weight (gr)	One week body weight (gr)	difference in body weight	One week feed intake (gr)	gr of feed needed to gain 1 gr of body weight
control	325	332	7	112	16
antibody	324	325	1	114	114

It will be apparent to those skilled in the art that a number of modifications and variations may be made without departing from the scope of the present invention as set forth in the appended claims.

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CLAIMS

1. Use of an antibody that binds to lipase for the manufacture of a medicament for the treatment of obesity in a mammal or an avian.

2. Use of an antibody that binds to lipase for the manufacture of a medicament for decreasing fat absorption in a mammal or an avian.

3. The use according to Claim 1 or Claim 2 wherein the mammal is a human, a primate, a monogastric or a ruminant.

4. The use according to Claim 1 or Claim 2 wherein the avian is a chicken, a turkey, a goose, a duck, a quail, a pheasant or a pigeon.

5. The use according to Claim 1 or Claim 2 wherein the antibody that binds to lipase is produced in avian eggs.

6. The use according to Claim 5 wherein the avian eggs are chicken, duck, goose, turkey, pheasant, quail or pigeon eggs.

7. The use according to Claim 5 wherein the antibodies are obtained from unfractionated whole eggs.

8. The use according to Claim 5 wherein the antibodies are obtained from the yolk of an egg without fractionation thereof.

9. The use according to Claim 5 wherein the antibodies are obtained by fractionating the egg yolk to form a protein concentrate or pure IgY (chicken immunoglobulin).

10. The use according to Claim 1 or Claim 2 wherein the antibody that binds to lipase is produced in an antibody-producing animal, a plant, a bacterium or a monoclonal antibody producing cell line.

11. The use according to any one of the preceding claims wherein the antibody is further processed to manufacture a medicament by freeze drying, spray drying or encapsulation.

12. The use according to Claim 11 wherein the encapsulation comprises protein coating, carbohydrate coating, liposomes, or other chemical processes that coat the antibody.

13. The use according to any one of the preceding claims wherein the medicament is administered orally.

14. The use according to any one of the preceding claims wherein the medicament is in powder or liquid form.

15. The use according to any one of Claims 1 to 14 wherein the medicament is incorporated into processed or prepared food.

16. Use of an antibody that binds to a gastro-intestinal enzyme for the manufacture of a medicament for decreasing absorption of nutrients such as protein, carbohydrates and lipids in animals or humans.

17. The use according to Claim 16 wherein the gastro-intestinal enzyme is selected from amylase, trypsin, chymotrypsin, proteases and other enzymes required for the absorption of nutrients.

18. The use according to Claim 16 or Claim 17 wherein the antibodies are derived from a producer animal or human wherein said producer animal or human has been immunized with an antigen wherein said antigen regulates a biochemical process in the gastro-intestinal tract.

19. A method for decreasing fat absorption in mammals and avians by feeding an antibody that binds lipase.

20. The method of Claim 19 wherein lipase is any enzyme that is needed for the hydrolysis of fat in order for it to be absorbed by the gastro-intestinal mucous.

21. The method of Claim 19 wherein the antibody binds to lipase therefore inhibiting its activity in the gastro-intestinal tract.

22. The method of Claim 19 wherein the lipase is of mammal, avian or plant origin.

23. The method of Claim 22 wherein the mammal is a human, a primate, a monogastric or a ruminant.

24. The method of Claim 22 wherein the avian is a chicken, turkey, goose, duck, quail, pheasant or pigeon.

25. The method of Claim 22 wherein plants include bacteria, mold and yeast.

26. The method of Claim 19 wherein antibody was produced in avian eggs.

27. The method of Claim 19 wherein the antibody is produced in other

commercial or laboratory antibody-producing animal including monoclonal, plant and bacteria produced antibodies.

28. The method of Claim 26 wherein avian compromises chicken, duck, goose, turkey, pheasant, quail or pigeon.

29. The method of Claim 26 wherein the antibodies are obtained from unfractionated whole eggs.

30. The method of Claim 26 wherein the antibodies are obtained from the yolk of an egg without fractionation thereof.

31. The method of Claim 26 wherein the antibodies are obtained by fractionating the egg yolk resulting in a protein concentrate or pure IgY (chicken immunoglobulin).

32. The method of Claim 19 wherein antibody produced as Claims 26-31 is kept as it was obtained or is further processed in order to freeze dry, spray dry or encapsulate.

33. The method of Claim 32 wherein encapsulation is such process that protects the antibody against changes that inactivate or disrupt the effectiveness of the antibody.

34. The method of Claim 32 wherein encapsulation methods are liposomes, protein coating, carbohydrate coating, other chemical processes that will coat the antibody.

35. The method of Claim 19 wherein the antibody or the antibody containing material is orally fed.

36. The method of Claim 35 wherein the orally fed antibody or the antibody containing material is fed by itself as powder or liquid form.

37. The method of Claim 36 wherein the powder or the liquid antibody or the antibody containing material is fed as part of a processed or prepared food.

38. The method of Claim 37 wherein processed or prepared food is any food where the antibody or the antibody containing material, in powder or liquid form, has been included as part of the formulation or recipe.

39. The method of Claim 38 wherein processed or prepared food is any food for human or animal consumption which include ready to eat, ready to mix, concentrate, additives, refrigerated and frozen food.

40. The method of Claim 19 wherein decreased fat absorption is due to the decreased lipase activity, therefore fat is excreted and not absorbed in the gastro-intestinal tract.

41. A method of transferring gastro-intestinal enzyme antibodies in animals and humans to other animals or humans in order to decrease absorption of nutrients such as protein, carbohydrates and lipids.

42. The method of Claim 41 wherein said gastro-intestinal tract enzyme is selected from amylase, trypsin, chymotrypsin, proteases and other enzymes required for the absorption of nutrients.

43. A method of transferring antibodies from an animal or human to other animals or humans in order to modify a biochemical process comprising:

administering to said animal or human an antibody containing substance wherein said substance was derived from a producer animal or human wherein said producer animal or human has been immunized with the antigen wherein said antigen regulates a biochemical process in the gastro-intestinal tract.